Claims

1. A method for chromatographic separation of a molecule, wherein a mobile phase and charged stationary phase are present and a charged amphipathic sugar polymer(s) is employed to modify the hydrophobic interaction between the molecule and said charged stationary phase.

2. A method for separating a molecule from a solution comprising the molecule and further components by hydrophobic interaction chromatography comprising applying the solution comprising the molecule to a charged stationary phase, and eluting the molecule from the stationary phase in a mobile phase, characterised in that:

(a) the charged stationary phase is non-covalently associated with a charged amphipathic sugar polymer(s), or the stationary phase comprises a charged amphipathic sugar polymer, and/or

(b) the molecule is non-covalently associated with a charged amphipathic sugar polymer(s).

3. A method according to claim 1 or claim 2, wherein the pH of the mobile phase is below the pI of the molecule (and thus the molecule carries a net positive charge).

4. A method according to claim 1 or claim 2, wherein the pH of the mobile phase is above the pI of the molecule (and thus the molecule carries a net negative charge).

5. A method according to claim 3 for separating a positively charged molecule from a solution comprising the molecule and further components by hydrophobic interaction chromatography comprising applying a solution comprising the molecule to a positively charged stationary phase which is non-covalently associated with a negatively charged amphipathic sugar polymer(s), and eluting the molecule from the stationary phase in a mobile phase.

6. A method according to claim 4 for separating a negatively charged molecule from a solution comprising the molecule and further components by hydrophobic interaction chromatography comprising applying a solution comprising the molecule to a negatively charged stationary phase which is non-covalently associated with a positively charged amphipathic sugar polymer(s), and eluting the molecule from the stationary phase in a mobile phase.

7. A method according to claim 3 for separating a positively charged molecule from a solution comprising the molecule and further components by mixed mode hydrophobic interaction/ion exchange chromatography comprising applying a solution comprising the molecule to a negatively charged stationary phase which is non-covalently associated with a positively charged amphipathic sugar polymer(s), and eluting the molecule from the stationary phase in a mobile phase.

8. A method according to claim 4 for separating a negatively charged molecule from a solution comprising the molecule and further components by mixed mode hydrophobic interaction/ion exchange chromatography comprising applying a solution comprising the molecule to a positively charged stationary phase which is non-covalently associated with a negatively charged amphipathic sugar polymer(s), and eluting the molecule from the stationary phase in a mobile phase.

9. A method according to any of the preceding claims, wherein the mobile phase comprises an amphipathic sugar polymer(s).

10. A method according to any of the preceding claims, wherein the mobile phase comprises a charged amphipathic sugar polymer(s).

11. A method according to claim 7 for separating a positively charged molecule from a solution comprising the molecule and further components by mixed mode hydrophobic interaction/ion exchange chromatography comprising applying a solution comprising the molecule to a negatively charged stationary

phase, and eluting the molecule from the stationary phase in a mobile phase comprising a negatively charged amphipathic sugar polymer(s).

12. A method according to claim 8 for separating a negatively charged molecule from a solution comprising the molecule and further components by mixed mode hydrophobic interaction/ion exchange chromatography comprising applying a solution comprising the molecule to a positively charged stationary phase, and eluting the molecule from the stationary phase in a mobile phase comprising a positively charged amphipathic sugar polymer.

13. A method according to any preceding claim, wherein the or an amphipathic sugar polymer is a cyclic sugar polymer.

14. A method according to claim 13, wherein the or an amphipathic sugar polymer is a glucosan or a derivative thereof.

15. A method according to claim 14, wherein the or an amphipathic sugar polymer is a cyclodextrin or derivative thereof.

16. A method according to claim 15, wherein the cyclodextrin is an α-cyclodextrin, a β-cyclodextrin, a γ-cyclodextrin, or a derivative thereof.

17. A method according to claim 16, wherein the cyclodextrin is a β-cyclodextrin derivative.

18. A method according to any one of claims 15 to 17, wherein the cyclodextrin derivative(s) is selected from the group comprising: cyclodextrin sulfate, sulfopropyl cyclodextrin, sulfobutylether cyclodextrin, cyclodextrin phosphate, carboxymethyl cyclodextrin, carboxyethyl cyclodextrin, succinyl hydroxypropyl cyclodextrin, quarternary ammonium cyclodextrin and 6-monodeoxy-6-monoamino cyclodextrin.

19. A method according to any of claims 1 to 12, wherein the amphipathic sugar polymer is a helical or linear sugar polymer.

20. A method according to any of claims 1 to 13 or claim 19, wherein the amphipathic sugar polymer is a fructosan or a derivative thereof.

21. A method according to claim 20, wherein the amphipathic sugar polymer is an inulin or a derivative thereof.

22. A method according to claim 21, wherein the inulin or inulin derivative has a degree of polymerisation of from about 3 to 500, 3 to 250 or 3 to 100, preferably from 3 to 50, more preferably from 10 to 50, yet more preferably from 15 to 40, further preferably from 20 to 30.

23. A method according to claim 21 or 22, wherein the inulin derivative is substituted with one or more charged group selected from the group consisting of: a sulfonyl group, sulfonylalkyl group, a phosphonyl group, a phosphonylalkyl group, a carboxyl group, a carboxyalkyl group, an alkyl-succinyl group, a quaternary ammonium group, an aminoalkyl group, an amino group, an alkylamino group and a dialkylamino group.

24. A method according to any one of claims 21 to 23, wherein the inulin derivative is a sulfonated inulin, carboxmethyl inulin, carboxethyl inulin, alkyl-succinyl-inulin, quarternary ammonium inulin, aminoalkyl inulin, amino inulin, alkylamino inulin or a dialkylamino inulin.

25. A method according to any one of claims 21 to 24, wherein the inulin derivative is derivatised by one or more type(s) of non-polar hydrocarbyl group.

26. A method according to any one of claims 21 to 25, wherein the inulin derivative is derivatised by one or more type(s) of non-polar hydrocarbyl group selected from the group comprising a linear alkyl derivative(s), branched alkyl

derivative(s) or a mixture of linear alkyl derivative(s) and branched alkyl derivative(s).

27. A method according to claim 21 or 22, wherein the or a inulin or inulin derivative is a compound of formula (I):

$$G(O\text{-}CO\text{-}NH\text{-}R^1)_a\text{-}[F(O\text{-}CO\text{-}NH\text{-}R^2)_b]_n \qquad (I)$$

wherein:

G is a terminal glucosyl unit in which one or more hydroxyl groups thereof may be substituted with a group or groups of formula (O-CO-NH-R^1);

R^1 is a charged substituent or a straight or branched chain saturated or unsaturated hydrocarbyl group having from 1 to 25 carbon atoms, said saturated or unsaturated hydrocarbyl group optionally being substituted with one or more charged substituents, and, where there is more than one (O-CO-NH-R^1) group on the glucosyl unit, each R^1 group may be the same or different;

a is an integer of from 0 to 4;

F is a fructosyl unit in which one or more hydroxyl groups thereof may be substituted with a group or groups of formula (O-CO-NH-R^2);

R^2 is a charged substituent or a straight or branched chain saturated or unsaturated hydrocarbyl group having from 1 to 25 carbon atoms, said saturated or unsaturated hydrocarbyl group optionally being substituted with one or more charged substituents, and, where there is more than one (O-CO-NH-R^2) group on the fructosyl unit, each R^2 group may be the same or different;

b is an integer of from 0 to 3 and from 0 to 4 for the terminal fructosyl unit;

n is an integer of from 2 to 499, preferably of from 2 to 249, 2 to 99, 2 to 49, 9 to 49, 14 to 39 or 19 to 29,

each unit of formula $F(O\text{-}CO\text{-}NH\text{-}R^2)_b$ may be the same or different from any other unit of formula $F(O\text{-}CO\text{-}NH\text{-}R^2)_b$; and

the average degree of substitution per glucosyl or fructosyl unit is from 0.02 to 3.0.

28. A method according to claim 27, wherein each group R^1 and R^2 is selected from alkyl, alkenyl and alkynyl groups having from 1 to 25, preferably 3 to 22, most preferably 3 to 18 carbon atoms.

29. A method according to claim 27 or claim 28, wherein one or more of the groups R^1 and R^2 is an alkyl group having from 1 to 25, preferably 3 to 22, most preferably 3 to 18 carbon atoms.

30. A method according to any one of claims 27 to 29, wherein one or more of groups R^1 and R^2 is an alkenyl or alkynyl group having from 1 to 25, preferably 3 to 22, most preferably 3 to 18 carbon atoms.

31. A method according to claim 27, wherein each alkyl group R^1 and R^2 is a linear alkyl group having from 1 to 25, preferably 3 to 22, most preferably 3 to 18 carbons or a branched alkyl group having from 3 to 25, preferably 3 to 22, most preferably 3 to 18 carbons.

32. A method according to any one of claims 27 to 31, wherein R^1 and/or R^2 is one or more charged group(s) selected from: a sulfonyl group, a phosphonyl group, a carboxy group, an alkyl-succinyl group, a quaternary ammonium group and an amino group, or is a hydrocarbyl group substituted with one or more charged group(s) selected from: a sulfonyl group, a phosphonyl group, a carboxy group, an alkyl-succinyl group, a quaternary ammonium group and an amino group.

33. A method according to any one of claims 27 to 32, wherein the inulin derivative of formula (I) is a sulfonylated inulin, carboxymethyl inulin, carboxyethyl inulin, alkyl-succinyl-inulin, quarternary ammonium inulin, aminoalkylinulin, amino inulin; alkylamino inulin or dialkylamino inulin.

34. A method according to any one of claims 27 to 33, wherein the average degree of substitution per glucosyl or fructosyl unit is from 0.02 to 3.0, preferably from 0.05 to 1.0, most preferably from 0.05 to 0.5.

35. A method according to any one of claims 27 to 34, wherein the compound of formula (I) is a polydisperse linear or slightly branched inulin N-alkylurethane.

36. A method according to any one of claims 27 to 35, wherein the compound of formula (I) is selected from the group consisting of inulin N-n-octyl-carbamates, inulin N-n-dodecylcarbamates and inulin N-n-octadecylcarbamates.

37. A method according to any preceding claim further comprising removal of amphipathic sugar polymer(s) from the eluate.

38. A method according to claim 37, wherein removal of amphipathic sugar polymer(s) from the eluate is by degradation of amphipathic sugar polymer(s) in the eluate.

39. A method according to claim 38, wherein degradation of the amphipathic sugar polymer(s) is by one or more of the following methods: chemical degradation, enzymic digestion, electromagnetic radiation, shear stress or heat.

40. A method according to claim 38 or 39, wherein degradation is in part or fully by enzymic digestion.

41. A method according to claim 40, wherein enzymic digestion is by glucosyltransferase, amylase, xylanase, exo-inulinase and/or endo-inulinase digestion.

42. A method according to claims 40 or 41, wherein enzymic digestion is by contacting the eluate with immobilised enzyme.

43. A method according to claim 42, wherein contacting is by passing eluate through a column containing immobilised enzyme.

44. A method according to any preceding claim, wherein the molecule for separation is hydrophobic or amphipathic.

45. A method according to any preceding claim, wherein the molecule for separation is a protein or a nucleic acid.

46. A method according to any preceding claim, wherein the molecule for separation is a charged protein and is separated from further protein(s).

47. A method according to any preceding claim, wherein the molecule for separation is a charged protein and is separated from further similarly charged protein(s).

48. A method according to any preceding claim characterised in that the charged stationary phase comprises a charged amphipathic sugar polymer(s).

49. A method for separating a molecule from a solution comprising a molecule and further components by hydrophobic interaction chromatography comprising applying the solution comprising the molecule to a charged stationary phase and eluting the molecule from the stationary phase in a mobile phase characterised in that the stationary phase comprises a charged amphipathic sugar polymer(s).

50. A method for separating a molecule from a solution comprising a charged molecule and further components by hydrophobic interaction chromatography comprising applying the solution comprising the molecule to an oppositely charged stationary phase, and eluting the molecule from the stationary phase in a mobile phase, characterised in that the stationary phase comprises a charged amphipathic sugar polymer(s).